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Synergism of CAY-1 with Amphotericin B and Itraconazole

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Key Words
Saponin · Synergism · Amphotericin B · Itraconazole · Additive-synergistic effect

Abstract
Background: CAY-1 is a fungicidal saponin from cayenne pepper whose mode of action differs from amphotericin B (AB) and itraconazole (IT). This work determined CAY-1 synergism with AB or IT. ***Methods:*** CAY-1 was purified and used in checkerboard microdilution studies where CAY-1 and AB or IT were mixed with nongerminated (NG) and germinating (G) conidia of three *Aspergillus* species and *Candida albicans.* Inhibition was visually determined at 24 and 48 h. ***Results:*** CAY-1 had predominantly additive-synergistic interaction with AB or IT against the *Aspergillus* NG and G conidia. Excellent synergy between CAY-1 and AB occurred at 24 and 48 h against *C. albicans.* Results suggest CAY-1 enhances AB and IT efficacy.

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Introduction

Discovery of novel, safe and effective antifungal drugs with novel modes of action is needed since extensive use of antifungal drugs has caused the emergence of resistant pathogens such as *Candida* [1, 2]. In addition, antifungal drugs can cause toxic or adverse drug reactions. For example, the polyene antifungal, amphotericin B (AB), has a broad range of activity but is limited in its use due to numerous adverse effects [3]. Saponins have surface-active properties which cause pores to form in microbial membranes leading to lysis, a mode of action which differs from those of AB or itraconazole (IT), an azole antifungal drug. CAY-1 is a fungicidal saponin (molecular weight 1,243 Da) present in the *Capsicum frutescens* fruit [4]. This work investigated potential in vitro synergism between CAY-1 and AB or IT at 24 and 48 h against nongerminated (NG) and germinating (G) conidia of several *Aspergillus* species and *Candida albicans.*

Materials and Methods

Purification of CAY-1

Aqueous extracts of cayenne pepper were freeze-dried then dissolved in deionized water (10% of the original volume) and added to a 400-gram preparative C_{18} column, 125 A, 55–105 μm (Waters

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Table 1. Synergism of CAY-1 with AB or IT against NG or G conidia of *Aspergillus* species and *C. albicans*

Fungus	Conidial type	Incubation time, h	Antifungal combination	FICI
A. flavus	NG	24	CAY-1 + AB	0.60
A. flavus	NG	48	CAY-1 + AB	2.00
A. flavus	G	24	CAY-1 + AB	1.50
A. flavus	G	48	CAY-1 + AB	1.38
A. flavus	NG	24	CAY-1 + IT	0.89
A. flavus	NG	48	CAY-1 + IT	0.88
A. flavus	G	24	CAY-1 + IT	1.50
A. flavus	G	48	CAY-1 + IT	1.25
A. fumigatus	NG	24	CAY-1 + AB	0.78
A. fumigatus	NG	48	CAY-1 + AB	0.82
A. fumigatus	G	24	CAY-1 + AB	0.66
A. fumigatus	G	48	CAY-1 + AB	0.74
A. fumigatus	NG	24	CAY-1 + IT	0.89
A. fumigatus	NG	48	CAY-1 + IT	0.64
A. fumigatus	G	24	CAY-1 + IT	0.66
A. fumigatus	G	48	CAY-1 + IT	0.86
A. niger	NG	24	CAY-1 + AB	0.81
A. niger	NG	48	CAY-1 + AB	2.00
A. niger	G	24	CAY-1 + AB	0.91
A. niger	G	48	CAY-1 + AB	0.99
A. niger	NG	24	CAY-1 + IT	0.83
A. niger	NG	48	CAY-1 + IT	0.66
A. niger	G	24	CAY-1 + IT	0.83
A. niger	G	48	CAY-1 + IT	0.58
C. albicans		24	CAY-1 + AB	0.22
C. albicans		48	CAY-1 + AB	0.32
C. albicans		24	CAY-1 + IT	2.0
C. albicans		48	CAY-1 + IT	2.0

Corp., Millford, Mass., USA) and eluted with a methanol (MeOH) step gradient [4]. CAY-1 was in the 75% MeOH eluate. MeOH was evaporated and the remaining liquid freeze-dried. CAY-1 was purified by reverse-phase high performance liquid chromatography using a Waters (Millford, Mass., USA) 25 × 100 mm C_{18} RCM column on an Applied Biosystems BioCad 700E (Foster City, Calif., USA). CAY-1 was detected by mass spectroscopy and lethality bioassays with *Aspergillus flavus* [1]. CAY-1 purity was confirmed by liquid chromatography and mass spectroscopy.

Checkerboard Microdilution Inhibition Studies

Synergy was measured by the checkerboard method [5]. AB or IT was diluted (1:2) along the rows of a sterile 96-well microtiter plate while CAY-1 was diluted (1:2) along the columns. NG and G conidia of *A. flavus, A. fumigatus,* and *A. niger* were separately tested in three separate runs per sample type. *C. albicans* was similarly tested. Fungal stock suspensions were adjusted to 10^2 conidia/well. Assays for all fungi but *A. niger* were performed in RPMI-1640 since earlier work showed CAY-1 was effective against *A. niger* only in 1% PDB [1]. End points were determined visually as the well having the lowest concentration of compounds with an absence of growth at 24 and 48 h. Results were expressed as the fractional inhibitory concentration index (FICI) [6]. FICIs of ≤ 0.5, 1.0, and >4.0 were considered indicative of synergy, additive activity, and antagonism, respectively.

Results

CAY-1 showed predominantly additive-synergistic interaction with AB or IT against the NG and G conidia of the tested *Aspergillus* species (table 1). CAY-1, at 24 and 48 h, enhanced AB activity against both the NG and G conidia of *A. fumigatus* and *A. niger* at the additive-synergy level. Less synergistic-additive interaction occurred against *A. flavus* where the predominant effect was indifferent. Excellent synergy between CAY-1 and AB occurred at 24 and 48 h against *C. albicans.* CAY-1 interacted indifferently with IT against *C. albicans.*

Discussion

CAY-1 was synergistic in vitro with both AB and IT against the NG and G conidia of the tested *Aspergillus* species at 24 and 48 h and was synergistic with AB, but not with IT, against *C. albicans.* AB interacts with ergosterol which causes increased membrane permeability for cations and cell death [7]. IT, a triazole, inhibits ergosterol synthesis which disrupts fungal membrane development [7] while saponins (e.g. CAY-1) affect membrane integrity. Combinations of antifungal compounds having differing modes of action could improve the likelihood of effective treatment of mycoses and reduce the risk of resistance in the target organism. Results indicate CAY-1 may enhance the efficacy of azole and polyene antifungals.

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